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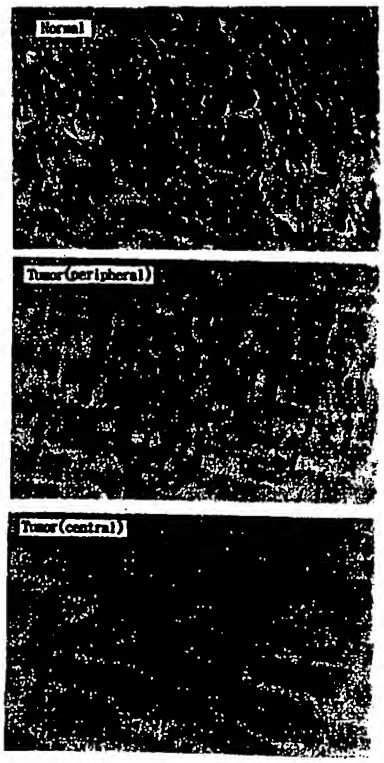
(54) Title: CANCER DIAGNOSIS METHOD USING CELL GROWTH INHIBITING AND CELL DIFFERENTIATION SPECIFIC SYG972 GENE AND GENOMIC DNA AND PROMOTER THEREOF

(57) Abstract

The present invention relates to a diagnosis method of cancer using SYG972 gene and to the genomic DNA and to a promoter regulating transcription of the gene. Expression of SYG972 gene is greatly reduced in breast cancer cells compared to normal breast cells. Therefore, the present invention provides a method of diagnosis method by using the gene of the present invention or its fragments as a probe. SYG972 genomic DNA according to the present invention is composed of 4 axons and 3 introns. Promoter is 3kb long from the transcription initiating site to the 5' direction. SYG972 genomic DNA and promoter can be used to design and screen drugs to promote or to inhibit apoptosis and differentiation of cells, especially to screen drugs to treat cancer, a disease wherein cell growth and differentiation is abnormal.



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CANCER DIAGNOSIS METHOD USING CELL GROWTH INHIBITING AND CELL DIFFERENTIATION SPECIFIC SYG972 GENE AND GENOMIC DNA AND PROMOTER THEREOF

TECHNICAL FIELD AND BACKGROUND ART

The present invention relates to a gene relating to cell differentiation (SYG972), amino acid sequence coded therefrom and their application to cancer diagnosis. The present invention also relates to a genomic DNA of SYG972 and to a new promoter.

Cell differentiation is the series of events involved in the development of a specialized cell having specific structure, functional, and biochemical properties. Cell differentiation occurs mainly at the time of fetus formation, but also occurs through out the entire life. Differentiating cells receive specific intracellular signals to participate in cell differentiation according to its program. During this process, expression of many different genes is regulated through a complex path. Genes that regulate cell differentiation are known to encode transcription-regulating factors including homeoproteins and cell cycle proteins. Most of the cell differentiation related genes, however, act on specific cell lines in a specific mode. Nevertheless, a common phenomenon relating to differentiation, that is, differentiation accompanying growth inhibition indicates that there exists a key regulator for cell differentiation. Accordingly, it is necessary to separate the functioning gene and elucidate its function.

One of the most prominent characteristics of cancer cells compared to normal cells is that cancer cells can amplify indefinitely. In order for normal cells to mutate to into cancer cells, the intracellular growth inhibiting genes must be deactivated. One of the representative example is the deactivation of the P53 gene that is found frequently in cancer cells. The product of the P53 genes is intra-nuclear transcription regulating factor for the normal cells, but mutation in this gene results in abnormal growth of the cells and which became cancer cells.

An example of a cancer regression gene closely related to cell differentiation is an erbA gene encoding thyroid hormone receptor. When red blood cell precursor cells are infected with an avian erythroblastosis virus, and the

function of the erbA gene is regulated abnormally, this cell differentiates not into red blood cell but into undifferentiated immortalized cancer cells. In other words, generation and progress of cancer cells are complex phenomena that accompany expression of many genes and also accompanies the blocking of cell differentiation. Therefore, it is very important to find factors regulating cell differentiation and growth inhibition in diagnosing and treating cancers.

Promoters, sites regulating the transcription of a gene, are generally located at 5' position of transcription start site. Gene expression is regulated depending on the transcription factor that binds this domain. In other words, the promoter of the gene determines regulation site for tissue specificity of a specific gene and changes in the expression according to the differentiation. Therefore, cloning a gene promoter and determining the sequence are very important in studying gene expression mechanism and finding gene expression regulating factors.

DISCLOSURE OF THE INVENTION

It is an object of the present invention to provide a cancer diagnosis method using SYG972 gene, its fragment and polypeptide derived therefrom.

It is another object of the present invention to provide a genomic DNA of SYG972.

Another object of the present invention is to provide a sequence for a new promoter.

Another object of the present invention is to provide a method to design and screen drugs by using a promoter of SYG972.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a Northern blot showing an expression of SYG972 mRNA in human tissue.

Figure 2A is a Northern blot showing an increase of SYG972 mRNA according to the differentiation of PC12 cell to nerve cell.

Figure 2B is a Northern blot showing an increase of SYG972 mRNA

according to the differentiation of C2C12 cell to muscle cell.

Figure 2C is a Northern blot showing an increase of SYG972 mRNA according to the differentiation of L6 cell to muscle cell.

Figure 3 is an *in situ* hybridization photograph showing the distribution of SYG972 in normal mouse tissue.

Figure 4 is an *in situ* hybridization photograph showing the distribution of SYG972 in human breast cancer tissue.

Figure 5 is a graph showing a degree of activation of SYG972 promoter.

DETAILED DESCRIPTION OF THE INVENTION

Terminology and techniques in the present application have the meanings that are generally used in the field of the present invention, unless otherwise specified. Also the references mentioned in the present application are the references which further explain the present invention and are incorporated by reference in the present application.

"Amino acid sequence", "polypeptide" or "protein" is not limited to the perfectly natural amino acid sequence.

"Sequence mutant" refers to an altered sequence due to substitution, deletion or addition of one or more bases in SYG972 and SYG 972 genomic DNA while maintaining a biological or immunological activity.

"Amino acid mutant" refers to an altered amino acid sequence due to substitution, deletion or addition of one or more amino acids derived from SYG972 while maintaining a biological or immunological activity.

"SYG972 derivative" refers to a gene wherein one or more of the base of SYG972 are altered while maintaining the biological characteristic of the protein that it encodes.

The present inventors have separated gene SYG972 using HL60 cell line as a model system. This cell line shows histo-pathological reading of acute promyelocytic leukemia, and especially has characteristics of differentiation by addition of various foreign factors such as TPA, DMSO and retinoic acid.

To isolate SYG972, mRNA was extracted from untreated HL60 cells and HL60 cells differentiated with TPA. cDNA was isolated by subtraction

hybridization (Fornace, A.J. (1988). DNA damage-inducible transcripts in mammalian cells. *Proc. Natl. Acad. Sci USA* 85, 8800-8804) and the resulted cDNA clone was identified by Northern blot and then isolated a gene that increases in the HL60 differentiation model. The sequence of SYG972 gene is listed as Sequence No. 1.

The database search based on the homology of the gene sequence resulted in two similar genes, Gadd45 and Myd118. Expression of Gadd45 (Growth Arrest and DNA Damaging inducible gene) increases upon X-ray or ultraviolet radiation or upon treatment with alkylating agent. Gadd45 has a characteristic of responding to DNA damaging (Fornace, A.J. (1988). DNA damage-inducible transcripts in mammalian cells. Proc. Natl. Acad. Sci USA 85, 8800-8804). Myd118 is a gene that increases upon Myeloid cell differentiation (Abdollahi, A. (1991). Sequence and expression of a cDNA encoding Myd118: a novel myeloid differentiation primary response gene induced by multiple cytokines. Oncogene 6, 165-17).

In spite of the homology among these genes, gene expression of SYG972 in the tissue is totally different with known Gadd45 or Myd118. Expression of Gadd45 or Myd118 is transiently increased by specific stimuli such as Myeloid cell differentiation or DNA damaging. On the other hand, SYG972 shows high expression rate in a variety of tissues. Therefore, SYG972 probably has an important role in the function of many different tissues in the absence of foreign stimuli.

In order to examine the function of SYG972 on cell differentiation, the expression behavior of SYG972 gene was investigated by a well-established differentiation model of various cells, and this investigation revealed that SYG972 increases upon the initiation of differentiation in PC12 cell line that differentiates into nerve cells and in C2C12 and L6 that differentiate to muscle cells.

Expression of SYG972 was observed in the heart, placenta, skeletal muscle, pancreas, gastrointestinal tract, thyroid, spinal cord, lymph node, trachea, adrenal, bone marrow, prost0ate, testis, ovary, small and large intestines. Among these, higher level of SYG972 expression was observed in the placenta, adrenal, testis and ovary. One thing to note is that the organs, in which the over expression was observed, are mostly mesoderms that have the same genetic

origin. Especially all of these organs function as steroid hormone synthesis and secretion. These facts indicate that SYG972 has an important role in a developing embryo, especially in the formation of mesodermal organs.

The amino acid sequence induced from SYG972 sequence is listed in Sequence No. 2.

The present invention relates to a cancer diagnosis method using a DNA having a sequence selected from the group consisting of Sequence 1, its fragment, its mutant, its derivatives, their fragments, and their allelomorphic mutant.

The present invention also relates to a cancer diagnosis method using a peptide which is coded from a DNA having a sequence selected from the group consisting of Sequence 1, its fragment, its mutant, its derivatives, their fragments, and their allelomorphic mutant or an antibody therefrom.

Expression of SYG972 gene is high in differentiated normal tissues but whereas it is inhibited upon canceration. This characteristic of SYG972 gene can be used in cancer diagnosis.

As an example, in a tissue of a breast cancer patient, mRNA of SYG972 was rarely observed around the cancer cells, but was highly expressed in the surrounding normal tissues. Especially among the cancer tissues, while a weak expression was observed at the boundary of cancer adjacent to the normal cells, there was no expression detected at the central cancer tissue. Therefore, SYG972 gene can be used as a diagnostic gene to distinguish cancer from normal cells.

As another example, SYG972 gene of the present invention is not expressed in B cells in the lymph node, but highly expressed specifically in T cells. Accordingly the SYG972 gene can be advantageously used in distinguishing B cells and T cells in searching the origin of cancer in various lymphomas.

The diagnosis method of the present invention, which is carried out with a tissue specimen isolated from a subject, can employ a well known hybridization method by using a DNA having a sequence selected from the group consisting of SYG972, its fragment, its mutant, its derivatives, their fragments and their allelomorphic mutant.

The diagnosis method of the present invention, which is carried out with a tissue specimen isolated from a subject, can employ a well known interaction method by using a peptide coded from a DNA having a sequence selected from

the group consisting of SYG972, its fragment, its mutant, its derivatives, their fragments and their allelomorphic mutant, or an antibody therefrom.

The present invention relates to the genomic DNA of SYG972 gene.

SYG972 genomic DNA according to the present invention comprises of 4 exons and 3 introns. The promoter is about 3 kb long from the transcription start site to the 5' direction.

Therefore, the present invention relates to a DNA, wherein the DNA has a sequence selected from the group consisting of:

a DNA comprising Sequence No. 3;

a fragment of a DNA having Sequence No. 3;

a mutant of a DNA having Sequence No. 3;

a derivative of a DNA having Sequence No. 3; and

an allelomorphic mutant of a DNA having Sequence No. 3.

The present invention also relates to a DNA having a sequence selected from the group consisting of

(a) a DNA sequence of comprising base No. 1 to 2973 of Sequence 3 or a fragment thereof; and

(b) a DNA sequence capable of hybridizing to (a),

wherein said DNA has a promoter activity.

The present invention relates to a method of designing and screening drugs using a SYG972 promoter.

Since SYG972 DNA plays an important role in cell differentiation, SYG972 genomic DNA and the promoter that regulates its expression can be used in designing and screening drugs that promote or inhibit cell apoptosis and differentiation. Especially these can be used as a useful tool in screening the drugs for the disease where cell differentiation and apoptosis occur abnormally, i.e., cancer, Alzheimer's disease or Parkinson's disease, and degenerative nervous diseases.

The present invention will be further illustrated by the following examples. It will be apparent to those have conventional knowledge in the field that these examples are given only to explain the present invention more clearly, but not limited to the examples given.

EXAMPLE 1. Isolation of SYG972 DNA

A HL60 cell line was cultured in RPMI (GibcoBRL) medium supplemented with 20 % bovine fetal serum, 100 unit/ml penicillin G sodium and 100 μg/ml streptomycin sulfate. Differentiated Hl60 cells were obtained by treating 5×10^{-8} M TPA (12-O-tetradecanoylphobol-13-acetate) in the medium for 3 days. Total RNA in the cell was isolated by an acid guanidinium thiocyanate phenol-chloroform method (Chomczynski, P., Sacchi, N. (1987)). Single-step method of RNA isolation by acid guanidinium thiocyanate phenol-chloroform extraction. (Anal. Biochem. 162, 156-159). The obtained RNA was dissolved in 20 μl sterilized distilled water for quantification by spectrophotometer and then only poly(A) DNA was isolated by oligo dT chromatography from which, a single strand cDNA was synthesized by using M-MLV reverse transcriptase. Differentiation specific cDNA was separated using hydroxyapatite chromatography. From the separated single strand cDNA, the second strand cDNA was synthesized and then cloned at EcoR I and Xho I sites of Uni-ZAP XR vector. (Schneder, C. et.al. 91988). Genes specifically expressed as growth arrest of mammalian cells. Cell 54, 787-793). Library of cloned vector was constructed by packing with packaging extract (Gigapack II packaging extract, Stratagene). Finally SYG972 gene was obtained from this library.

By determining DNA sequence, SYG972 gene was identified as 1066 bp long. Among the sequence, the open leading frame that can be translated into protein is 480 bp long (Sequence No. 1). SYG972 amino acid sequence estimated from the DNA sequence is listed as Sequence No. 2.

EXAMPLE 2. Expression behavior of SYG972 in human tissue

To investigate the level of expression of SYG972 in human tissue, total RNA was extracted from human tissue and analyzed by Northern blot hybridization (Sambrook, J. et. al. (1989). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press). To a membrane (Clontech) blotted with extracted RNA from human tissue, SYG972 DNA probe marked with α-^{32}P-[dCTP] was hybridized and reacted for 24 hours and washed with 2 x SSC, 0.1 % SDS solution for 10 minutes at room temperature and for 30 minutes at 50 °C. The washed membrane was exposed at -70 °C for 1 week on an x-ray film and

developed. The result is shown in Figure 1.

Expression of SYG972 varied for different tissues. Expression of SYG972 was observed in the heart, placenta, skeletal muscle, pancreas, gastrointestinal tract, thyroid, spinal cord, lymph node, trachea, adrenal, bone marrow, prostate, testis, ovary, small and large intestines. Among these, over expression was observed in the placenta, adrenal, testis and the ovary.

EXAMPLE 3. Expression behavior of SYG972 in various differentiation models

To investigate the quantitative change of SYG972 mRNA during cell differentiation, three different cell differentiation models were used. PC12 cells which are pheochromocytoma, have a characteristic of differentiating to nerve cells by NGF (nerve growth factor), and C2C12 and L6 which are mouse myoblasts and white mouse myoblast respectively, have a tendency to differentiate to muscle cells by a stimulus inducing differentiation. PC12 cells were cultured in 10 % horse serum (GibcoBRL) supplemented with 5 % bovine fetal serum, 100 unit/ml penicillin G sodium and 100 μg/ml streptomycin sulfate. To obtain RNA from differentiated PC12 cells, 50 ng/ml of NGF (Gibco BRL) was added in the medium. After a few hours, differentiated cell morphology was identified under microscope and collected at different time intervals.

C2C12 and L6 cells were cultured in 10 % bovine fetal serum supplemented with 100 unit/ml penicillin G sodium and 100 μg/ml streptomycin sulfate. To differentiate these cells, the medium was exchanged with DMEM supplemented with 2 % horse serum and 5 % horse serum, respectively. After a few hours, differentiated cell morphology was identified under microscope.

Total RNA was extracted from each sample as described in Example 1. Thirty micrograms of total RNA was mixed with the same volume of RNA loading buffer (50 % formamide, 6.2 % formaldehyde, 20 mM MOPS (3-[N-morpholino]propanesulfonic acid), 5 mM sodium acetate, 1 mM EDTA) and denatured for 10 minutes at 65 °C. All of the samples were electrophoresed in 1.2 % formaldehyde gel for 1 hour at 100 V, separated RNA was transferred into Nylon membrane for 48 hours by a capillary method. The membrane was finally cross-linked using UV cross-linker (UVP500; Hoefer, 120,000 μJ/cm^2). To prepare SYG972 cDNA probe marked with radioisotope, SYG972 cDNA was cut from

vector using EcoR I and Xba I restriction enzymes, marked with 50 μCi α-^{32}P-[dCTP] per 25 ng DNA by using Radprime labeling kit (Gibco BRL), and separated by using NucTrap paobe separation column.

The Nylon column prepared above was cultures in hybridization buffer (50 % formamide, 5 x SSPE, 5 x Denhardt solution, 0.01 % SDS, 1 mg/ml denatured salmon sperm DNA). To this, separated probe was added and hybridized for 24 hours. The membrane was washed with primary washing solution (2 x SSC, 0.1 % SDS) at room temperature for 10 minutes, with secondary washing solution (0.1 x SSC, 0.1 % SDS) for 30 minutes at 50 °C, and exposed at −70 °C for 1 week on an x-ray film and developed. As shown in Figures 2A, 2B, and 2C, SYG972 RNA increased meaningfully as the degree of cell differentiation progressed. As PC12 differentiated to nerve cells, RNA levels increased 7 days after induction of differentiation. In the case C2C12 and L6 cells differentiated to muscle cells, large amount of RNA was found 1 day after differentiation induction.

EXAMPLE 4. Expression of SYG972 in mouse tissue

SYG972 gene expression level was investigated by using *in situ* hybridization method developed by Braissant and co-workers (Braissant, O. et. al. (1996)). Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in an adult rat. Endocrinology 137, 354-366). In other words, antisense-DIG SYG972 RNA probe was prepared by cutting pBluescript II vector containing SYG972 cDNA with EcoR I and treated with DIG-11UTP. Mouse tissue specimen for analysis was obtained from anaesthetized 5 week old mouse and fixed for 24 hours in 4 % formaldehyde-PBS and dehydrated continuously in 70 %, 95 % and 100 % Xylene. Dehydrated specimen was solidified in parablast, cut in 5 μm width and adhered to ProbeOn plus slide (Fisher). Adhered specimen was hybridized with antisense probe after pre-treatment. Non-specifically bound probes were washed through post-treatment. To identify the probe in the specimen, colorization reaction was induced by using DIG specific antibody (Boeringer Mannheim) and NBT (Nitroblue tetrazolium), BCIP (5-bromo-4-chloro-3-indolylphosphate) as substrates. The tissue whose *in situ* hybridization is finished were observed under microscope and photographed. Representative results are shown in Figure 3 and Table 1. DIG

specific antibody attached with alkali phosphorylation enzyme used in identifying RNA marked with DIG formed dark blue precipitation with NBT and BCIP Therefore, dark blue region in the photograph is where the expression of SYG972 was high. As can be seen from Figure 3 and Table 1, SYG 972 gene is highly expressed in luteum of the ovary, Sertoli cells and germ cells in the testis, epidermal cells of the inner layer of Fallopian tube, gland cell of the prostate and uterus, T cell in lymph node, nerve cells in the cerebrum and spinal cord, myeloid cells of the bone marrow and megakaryocyte. Especially the expression level increased as leteum developed in Follicle cells that composed of leteum in the ovary, meaning that SYG972 is related with cell differentiation as described above. In the lymph node, expression was not found in the B cell, but was high specifically in T cell. Also in the cerebrum and spinal cord, SYG972 gene was specifically expressed in the nerve cells only.

Table 1

Tissue	Cell type	Degree of expression
Cerebrum	Cerebrum cortex nerve cell	+++
	Hippocampus nerve cell	+++
	Hypothalamus	+++
Cerebellum	Purkenje cell	++
Spinal cord	Nerve cell	+++
Hypophyseos		+++
Kidney	Peroximal tubule	++
	Distal tube	-
Liver	Hepatocyte	-
Lung	Alveolar cell	+
Pancreas	Acinus	-
	Islet	-
Heart	Cardiac cell	+
White fat cell		++
Brown fat cell		-
Spleen	White pulp	++
	Red pulp	-
Lymph node	T cell	+++
	B cell	-
Testis	Spermatogonia	+++
	Sertoli cell	+++
	Leydig cell	-
Ovary	Oocyte	-
	Follicular cell	+++
	Luteum	+++
	White luteum	+
Uterus	Endometrial gland	++
	Myometrium	-
	Fallopian tube	+++
Osteoid tissue	Chondrocyte	+++
	Ganglion	+++

EXAMPLE 5. Expression of SYG972 in human breast cancer tissue

Using *in situ* hybridization method as in Example 4, breast cancer cells from different patients were collected to investigate the expression of SYG972 mRNA. Results are shown in Figure 4 and Table 2. SYG972 mRNA was scarcely observed in tumor cells whereas it was highly expressed in the surrounding normal cells. Especially among the cancer tissues, weak expression was observed at the boundary of cancer which adjacent to the normal cells, but SYG972 was not expressed in the center of the cancer tissue at all.

Table 2

Type	Tissue site	Degree of expression
Carcinoma	Normal	++
	Tumor (exterior)	-
	Tumor (central)	-
Carcinoma	Normal	+++
	Tumor (exterior)	+
	Tumor (central)	-
Carcinoma	Normal	+++
	Tumor (exterior)	++
	Tumor (central)	-
Musinous Carcinoma	Normal	+++
	Tumor (exterior)	++
	Tumor (central)	-
Carcinoma	Normal	+++
	Tumor (exterior)	++
	Tumor (central)	+
Carcinoma	Normal	+++
	Tumor (exterior)	++
	Tumor (central)	-

EXAMPLE 6. Isolation of SYG972 gene genomic DNA

To isolate SYG972 gene genomic DNA from Lamda EMBL3 SP6/T7 human genome library (Clontech), 5 x 10^6 pfu phage was inoculated to K802 host bacteria and spread on 10 separate 150 mm petri-dishes. After observing bacterial lysis by phage, it was transferred on Nylon membrane, denatured according to the manufacturers' manual and cross-linked. A probe to screen SYG972 gene genomic DNA was obtained by marking total cDNA of SYG972 DNA gene with α-^{32}P-[dCTP] by a random primer labeling method. The thusly prepared cDNA probe was hybridized by Southern blot hybridization, washed and exposed on x-ray film for 1 week to determine the existence of SYG972 genomic DNA. With 13 positive clones obtained from the first screening, 2nd and 3rd screening were performed using the same procedures finally obtaining a single clone containing 11 kb long insert. The obtained clone was cut by BamH I restriction enzyme, electrophoresed in 0.8 % agarose gel to obtain 4 kb DNA fragment by performing Southern blot hybridization by using cDNA probe marked with radioisotope. The thusly obtained fragment was cloned into pUC19 plasmid vector, DNA sequenced to verify that it is SGY972 gene genomic DNA. DNA

sequence of SYG972 gene is listed as Sequence No. 3.

SYG972 genomic DNA is composed of 4 exons and 3 introns, the sequence that connects exons coincides accurately with SYG972 cDNA sequence. The promoter comprises base No. 1 to base No. 2973. Exon 1 is base No. 2974 to base No. 3036, Exon 2 is base No. 3394 to base No. 3495, Exon 3 is base No. 3629 to base No. 3901, and Exon 4 is base No. 3929 to base No. 4444.

EXAMPLE 7. Determination of activity of SYG972 (Gadd45-γ) promoter

To determine the activity of SYG972 promoter, the promoter region was cloned into pGL-basic (Promega) vector. The Cloned promoter site starts ca. 80 bp upper segments of the protein synthesis signal (ATG codon) and has a 1.2 kb size towards the 5' direction. This vector was co-transferred with pRL-TK (Promega) vector into monkey kidney cell line, COS-7 cell line using LipofecAmine Plus (GibcoBRL). Twenty four hours after the transfer, cells were sonicated to obtained a protein extract. Using 20 μl of the extract, activities of Firefly luciferase and Renila luciferase were determined by using Luminometer. As a control group to determine SYG972 promoter activity, pGL-basic vector without promoter and pGL-control vector with SV40 promoter and enhancer were used. The error that can be caused by DNA transfer and amount of protein were calibrated with Renila luciferase activity.

As shown in Figure 5, SYG972 promoter has an activity as a promoter in monkey kidney cell line.

According to the present invention, SYG972 genomic DNA can be used in cancer diagnosis and treatment and in diagnosing cell differentiation related disease. Also the SYG972 gene promoter according to the present invention can be used in designing and screening drugs for cancer and degenerative nervous disease.

CLAIMS

What is claimed is:

1. A method of diagnosing a cancer of a tissue specimen isolated from a subject using a hybridization method, which comprises utilizing at least one DNA probe selected from the group consisting of:
   a DNA having Sequence No. 1;
   a fragment of a DNA having Sequence No. 1;
   a mutant of a DNA having Sequence No. 1;
   a derivative of a DNA having Sequence No. 1; and
   an allelomorphic mutant of a DNA having Sequence No. 1.
2. A method according to Claim 1, wherein the cancer is breast cancer.
3. A breast cancer diagnosis kit comprising at least a DNA selected from the group consisting of:
   a DNA having Sequence No. 1;
   a fragment of a DNA having Sequence No. 1;
   a mutants of a DNA having Sequence No. 1;
   a derivative of a DNA having Sequence No. 1; and
   an allelomorphic mutant of a DNA having Sequence No. 1.
4. A method of diagnosing a cancer by reacting a tissue specimen isolated from a subject with at least one peptide probe which is coded from the DNA selected from the group consisting of:
   a DNA having Sequence No. 1;
   a fragment of a DNA having Sequence No. 1;
   a mutant of a DNA having Sequence No. 1;
   a derivative of a DNA having Sequence No. 1; and
   an allelomorphic mutant of a DNA having Sequence No. 1.
5. A method according to Claim 1, wherein the cancer is breast cancer.
6. A breast cancer diagnosis kit comprising at least one peptide which is coded from a DNA selected from the group consisting of:
   a DNA having Sequence No. 1;
   a fragment of a DNA having Sequence No. 1;
   a mutant of a DNA having Sequence No. 1;

a derivative of a DNA having Sequence No. 1; and

an allelomorphic mutant of a DNA having Sequence No. 1.

7. A DNA, wherein the DNA has a sequence selected from the group consisting of:

a DNA having Sequence No. 3;

a fragments of a DNA having Sequence No. 3;

a mutant a DNA having of Sequence No. 3;

a derivative of a DNA having Sequence No. 3; and

an allelomorphic mutant of a DNA having Sequence No. 3.

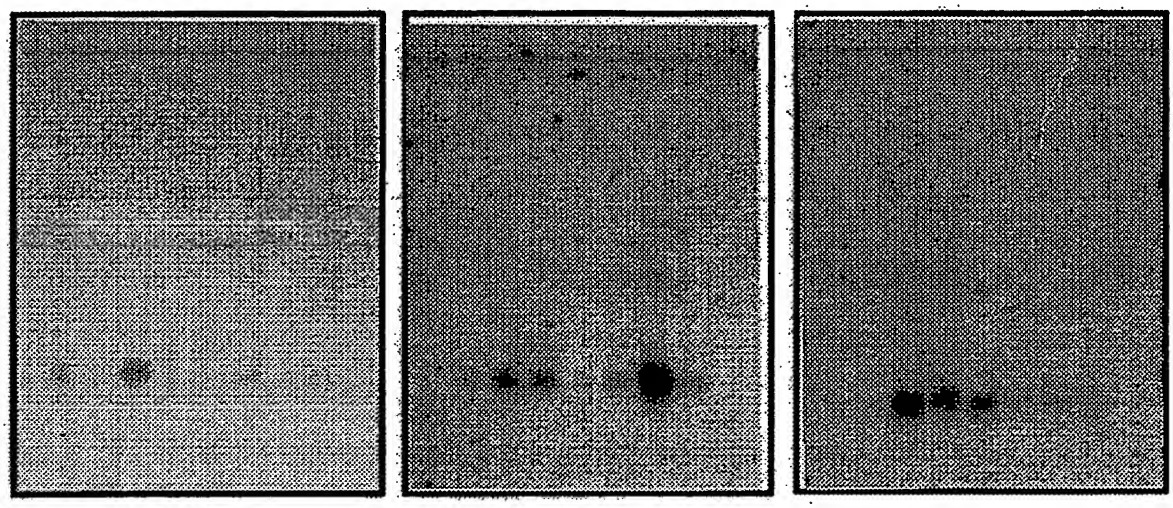
8. A DNA having a sequence selected from the group consisting of

(a) a DNA sequence comprising base No. 1 to 2973 of Sequence 3 or a fragment thereof; and

(b) a DNA capable of hybridizing to (a),

wherein said DNA has a promoter activity.

FIG. 1



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1. Heart
2. Brain
3. Placenta
4. Lung
5. Liver
6. Skeletal muscle
7. Kidney
8. Pancreas
9. Stomach
10. Thyroid
11. Spinal cord
12. Lymph node
13. Trachea
14. Adrenal gland
15. Bone marrow
16. Spleen
17. Thymus
18. Prostate
19. Testis
20. Ovary
21. Small intestine
22. Colon
23. Peripheral blood leukocyte

FIG. 2A

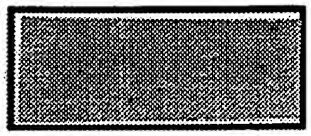
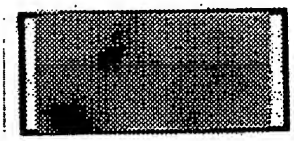


FIG. 2B

FIG. 2C



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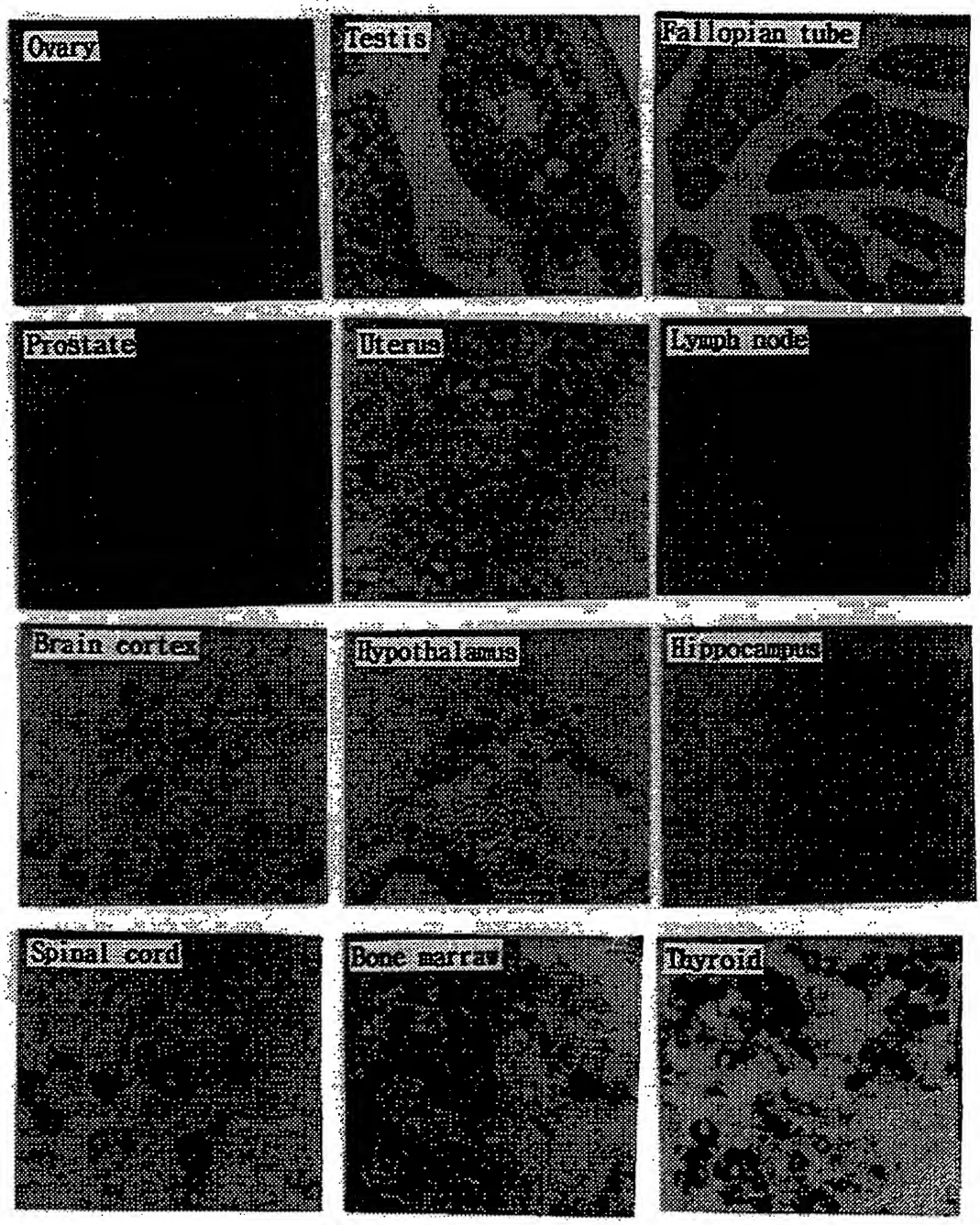
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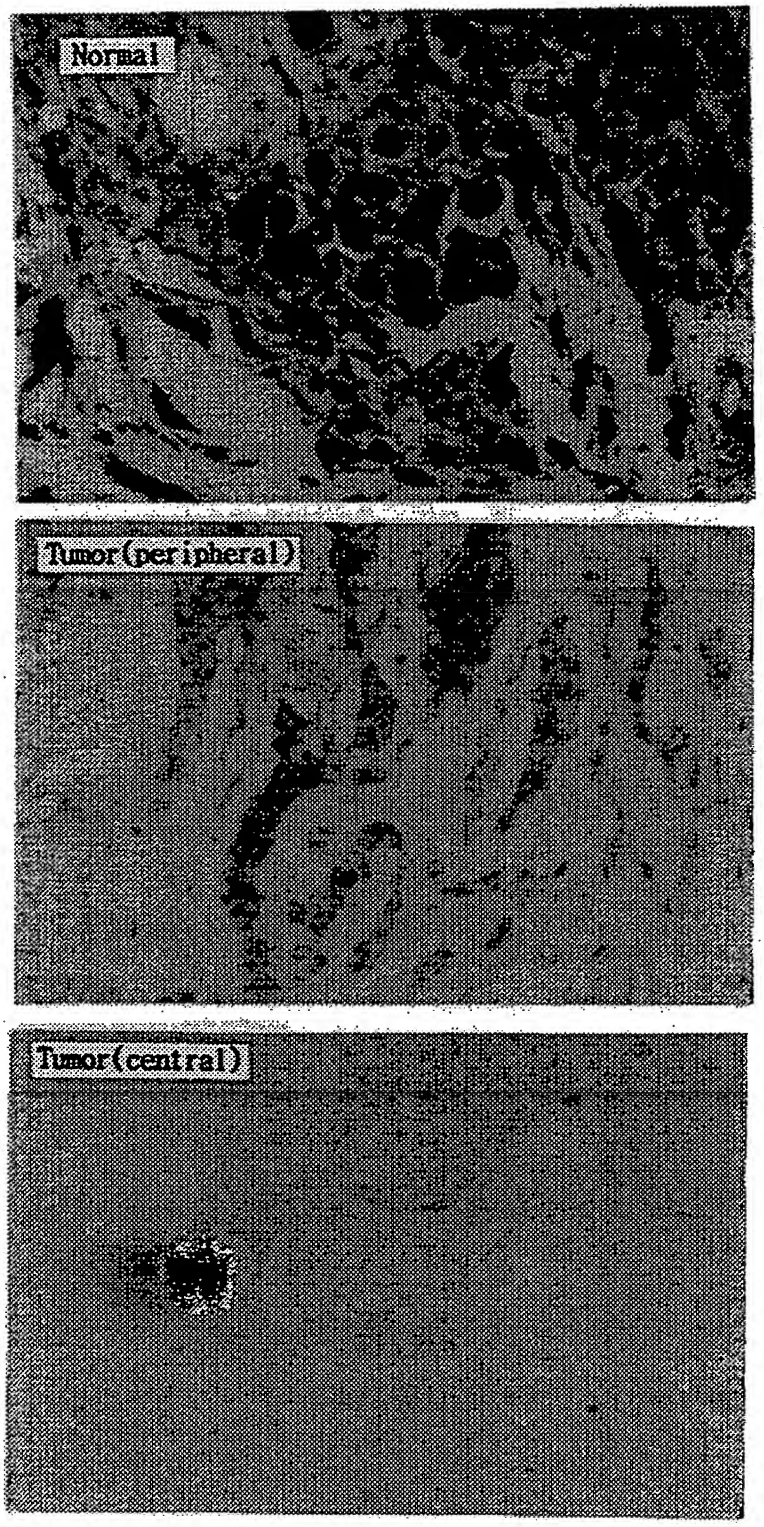
Fallopian tube
Prostate
Uterus
Lymph node
Brain cortex
Hypothalamus
Hippocampus
Spinal cord
Bone marrow
Thyroid

FIG. 4



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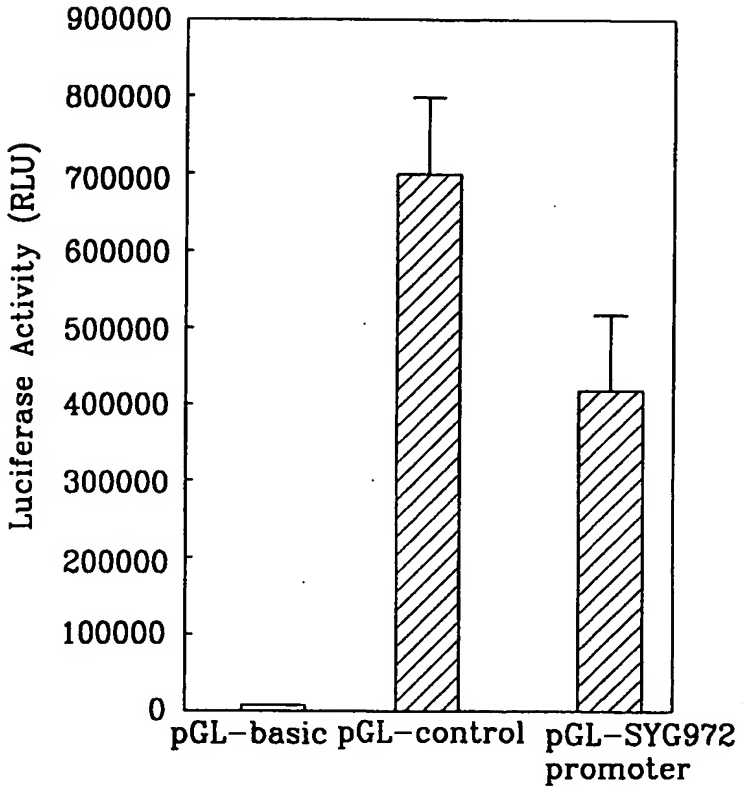
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00756

A. CLASSIFICATION OF SUBJECT MATTER

IPC[7]: C 12 Q 1/68, C 07 K 14/435, C 12 N 15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: C 12 Q 1/68, C 07 K 14/435, C 12 N 15/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS, STN-registry

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/39427 A1 (TRUSTEES OF DARTMOUTH COLLEGE) 12 December 1996 (12.12.96), page 2, lines 30, 31, page 29, line 22 - page 32, line 10.	1,4,7
X	EP 0787798 A2 (OTSUKA PHARMACEUTICAL CO.) 06 August 1997 (06.08.1997), abstract; page 5, lines 10-14; page 6, lines 49-53; SEQ ID NO: 3.	1,4,7
A	WO 94/11533 A1 (THE GOVERNMENT OF THE UNITED STATES, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 26 May 1994 (26.05.94) page 1, lines 5-11, lines 32-35; claims.	1-8
X	MUTSUHIRO et al. A Family of Stress-Inducible GADD45-like Proteins Mediate Activation of the Stress-Responsive MTK1-MEKK4 MAPKKK. Cell, November 1998, Vol. 95, pages 521-530.	7

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
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„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed
„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 February 2000 (18.02.00)	04 April 2000 (04.04.00)

Name and mailing adress of the ISA/AT	Authorized officer
Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/200	Mosser Telephone No. 1/53424/437

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR 99/00756

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
WO	A1	9639427	12-12-1996	EP	A1	832117	01-04-1998
				US	A	5871961	16-02-1999
				US	A	5871960	16-02-1999
				US	A	6027914	22-02-2000
				US	A	5882894	16-03-1999
				US	A	6020155	01-02-2000
				WO	A1	9310230	27-05-1993
				US	A	5795752	18-08-1998
EP	A2	787798	06-08-1997	CA	AA	2197020	10-08-1997
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				US	A	5808031	15-09-1998
WO	A1	9411533	26-05-1994	AU	A1	56059/94	08-06-1994
				US	A	5616463	01-04-1997
				US	A	5858679	12-01-1999